

number of repetitions that need to be carried out by the fluid handler subsystem can be cartridge specific and can be automatically ascertained by the cartridge reader from the information encoded in the machine-readable indicia affixed/incorporated onto the cartridge. The number of repetitions may be predetermined through empirical results but may also be determined in-situ through the use of one or more sensors adapted and configured to measure the degree of mixing of the reagent(s) and sample fluid; e.g., use of optical sensors (transmittance or reflectance), electrical sensors (impedance, conductance, resistance, and the like).

[0274] The sample fluid slugs are now moved into their detection chambers **2550A** and **2550B** by operating the pump with air vent valve **2422** and waste chamber vent valve **2442A** open until the sample slug is detected at sensor **7** and by operating the pump with air vent valve **2422** and waste chamber vent valve **2442B** open until the sample slug is detected at sensor **8** (FIG. 26, panels **2607-2608**). The sample slugs are incubated in the detection chambers to allow constituents of the sample (e.g., labeled binding reagents, analyte, control analyte, etc.) and immobilized binding reagents within the detection chamber to bind to form binding complexes in the detection chamber. Preferably, a mixing operation is employed to enhance the rate of these binding reactions. Preferably, mixing is achieved by moving the fluid slugs back and forth in the detection chamber by a process analogous to that described for reconstituting the reagent pill (optionally, using sensors **1**, **2**, **11** and **12** to provide stopping points in each direction). The aspirate and dispense operations are repeated a predetermined number of times, or until the degree of mixing desired has been achieved/detected. After completion of the incubation step, the air and waste chamber vent valves are used to draw the slugs out of the detection chambers and into waste chambers **2540A** and **B** (FIG. 26, panels **2609-2610**).

[0275] Preferably (as shown), the assay process includes a wash step for removing sample and unbound labeled reagents from the detection chamber. The wash uses a wash reagent (preferably, a buffered solution, more preferably comprising a non-ionic surfactant such as Triton X-100 and most preferably comprising an ECL coreactant such as TPA or PIPES) stored in reagent chamber **A 2530A**. If the wash reagent is in a reagent module (preferably, ampoule) and the module hasn't been opened, it is opened now. Optionally, the remaining sample fluid is first routed back into the sample chamber to prevent contamination of the wash reagent: first wash reagent is drawn from reagent chamber **A 2530A** into one of the sample conduit branches by operating the pump to apply negative pressure with reagent chamber **A** vent valve **2432A** and the corresponding waste chamber vent valve **2442A** or **B** open (and, preferably, overcoming a capillary break provided by a z-transition in the reagent conduit); then excess sample is drawn into the sample chamber by operating the pump to apply positive pressure to the waste chamber vent with the sample chamber vent valve open (FIG. 26, panels **2611-2612**). Wash reagent is then drawn from reagent chamber **A 2530A**, through detection chambers **2550A** and **2550B** and into waste chambers **2540A** and **2540B** by operating the pump with reagent chamber **A** vent valve **2432A** and waste chamber vent valves **2442A** and/or **2442B** (simultaneously or sequentially) open (FIG. 26, panels **2613-2616**). As shown, in particularly preferred embodiments, the wash fluid may be segmented, i.e., broken up by one or more slugs of air. It has been observed that

wash fluid alternating with air within the detection chambers increases the effectiveness of the clean cycle. Segmenting the wash fluid can be accomplished by periodically and temporarily opening the air vent valve **2422** and simultaneously closing the reagent chamber **A** vent valve **2432A** so that air is drawn into the sample conduit. Timing and duration of these operations would dictate the size and frequency of the air slugs introduced into the segmented wash fluid slug.

[0276] In the two step format, one or more labeled detection reagents may be incubated in the detection chambers in an additional incubation step. Preferably, the detection reagent solution is prepared by reconstituting a dry reagent pill comprising the detection reagents with an assay diluent contained within reagent chamber **B 2530B**. If the assay diluent is in a reagent module (preferably an ampoule) and it is not already broken, it is broken now. The assay diluent is drawn into elongated reagent conduit **2535** by aspirating at one of the waste chamber vents while opening reagent chamber **B** vent valve **2432B** until the assay diluent reaches sensor **13** (FIG. 26, panel **2617**). A defined volume of assay diluent is prepared by closing reagent chamber **B** vent valve **2432B** and opening air vent valve **2422** and continuing to aspirate at the waste chamber vent; reconstitution of the dry reagent in the elongated reagent conduit is promoted by alternating the pump between positive and negative pressure so as to move the slug back and forth over the dry reagent pill (FIG. 26, panel **2618-2619**). In a process analogous to the introduction of sample to the detection chambers, the slug of detection reagent solution is i) distributed between the sample conduit branches **2515 A** and **B**, ii) introduced to the detection chambers (**2550 A** and **B**), incubated in the detection chambers while moving the slugs back and forth in the chambers to increase the rate of the binding of the detection reagents to immobilized assay components in the chambers, and iii) expelled from the detection chambers to the waste chambers **2540 A** and **B** (FIG. 26, panels **2620-2622**). Optionally, residual detection reagent solution is washed from the detection chambers **2550A** and **B** by aspirating at the waste chamber vents with the reagent chamber **B** vent valve **2432B** open (and, preferably, alternating opening reagent chamber **B** vent valve **2432B** and air vent valve **2422** so as to segment the fluid stream) and then with air vent valve **2422** continuously open to draw the excess assay diluent into the waste chambers (FIG. 26, panels **2623-2625**). Alternatively, washing can be accomplished using the wash reagent by repeating the steps in panels **2613-2616**.

[0277] To provide an appropriate environment for the ECL measurement, detection chambers **2550A** and **2550B** are filled with the wash reagent (which preferably, is an ECL read buffer comprising an ECL coreactant). Accordingly, wash reagent is introduced into the detection chambers by operating the pump with reagent **A** chamber vent valve **2432A** and waste chamber vent valves **2442A** and/or **2442B** open so as to aspirate wash reagent into sample conduit branches **2515A** and **2515B**. Operating the pump with air vent valve **2422** and waste chamber valves **2442A** and/or **2442B** open introduces slugs wash fluid into the detection chambers (FIG. 16, panels **2628-2631**). The above assay is described for a two-step assay that employs two binding steps. An analogous protocol may be used for a one step protocol with one binding step, preferably, by omitting the steps in FIG. 26, panels **2617-2625**. In the one step format,